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Negative results

Contribution of *VPS35* genetic variability to LBD in the Flanders-Belgian population

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Abstract

VPS35 was recently identified as a novel autosomal dominant gene for Parkinson disease. In this study, we aimed to determine the contribution of simple and complex *VPS35* variations to the genetic etiology of the spectrum of Lewy body disorders (LBD) in a Flanders-Belgian patient cohort ($n = 677$). We identified 3 novel missense variations in addition to 1 silent and 1 intronic variation predicted to activate a cryptic splice site, but no copy number variations. Despite the absence of these rare variations in the control group ($n = 800$), we could not attain convincing evidence for pathogenicity by segregation analysis or in silico predictions. Hence, our data do not support a major role for *VPS35* variations in the genetic etiology of Lewy body disorders in the Flanders-Belgian population.

Keywords: Lewy body brain disorders; *VPS35*; Genetic etiology

1. Introduction

The first Parkinson disease (PD) gene identified by next generation sequencing was the vacuolar protein sorting 35 gene (*VPS35*). Two research groups independently identified VPS35 p.D620N as the probable disease-causing variation in large autosomal dominant PD pedigrees using whole exome sequencing (Vilarino-Guell et al., 2011; Zimprich et al., 2011). To date, no other pathogenic *VPS35* variations have been reported. We aimed to determine the role of *VPS35* genetic variability to the etiology of Lewy body disorders (LBD) in a well-characterized Flanders-Belgian LBD cohort.

2. Methods

We performed in-depth sequence analyses of all coding and noncoding *VPS35* exons ($n = 17$) and exon-intron boundaries in an extended LBD patient group comprised of PD ($n = 520$), Parkinson disease with dementia (PDD; $n = 72$), and dementia with Lewy bodies (DLB; $n = 85$) patients, living throughout Flanders. Novel intronic or synonymous variations predicted to have an effect on splicing and novel missense variations were validated in patients and

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Table 1
Novel *VPS35* missense variations and silent or intronic variations predicted to alter splicing

Patient	Exon/intron	Genomic position	Predicted cDNA	Predicted protein	PolyPhen-2	SNAP	SIFT	FSPLICE	Controls
d11720	Exon 6	g.10155G>A	c.585G>A	p.V195	/	/	/	− (6.38 vs. TH 6.099)	0/800
d2432	Intron 10	g.16691G>A	c.1161–70G>A	/	/	/	/	− (5.67 vs. TH 4.175)	0/800
d2542	Exon 14	g.26102T>C	c.1679T>C	p.I560T	− (0.998–0.27–0.99)	+	− (0.02)	+	0/800
d2700	Exon 14	g.26219A>G	c.1796A>G	p.H599R	+	+	+	+	0/800
d12436	Exon 14	g.26242A>G	c.1819A>G	p.M607V	+	+	+	+	0/800

Numbering of variations at the level of genomic DNA is relative to GenBank Accession Number NC_000016.9, cDNA to NM_018206.4, and amino acid substitutions are numbered according to GenPept Accession Number NP_060676.2. "-" predicted to be (probably) damaging; "+" predicted to be benign. SIFT scores < 0.05 suggest pathogenicity. The first PolyPhen-2 score indicates the probability of a deleterious effect, the second and third score the prediction's sensitivity and specificity. FSPLICE scores and the threshold (TH) value are shown.

genotyped in 800 control individuals using iPLEX gold chemistry (Sequenom Inc., CA, USA) on the Sequenom MassARRAY platform or direct sequencing. Additionally we performed *VPS35* dosage analyses of the extended patient group using multiplex amplicon quantification. Materials and methods are described in more detail in the Supplementary data.

3. Results

We identified 28 novel variations (Table 1, Supplementary Table 3), 3 of which predicted the amino acid substitutions p.I560T, p.H599R, and p.M607V at evolutionary strongly conserved codons (Supplementary Fig. 1) and were only observed in patients. Polyphen-2, SNAP, and SIFT predicted benign effects for p.H599R and p.M607V. Predictions for p.I560T were inconsistent and this variation was also present in an unaffected sibling (AAI, 89 years old) of the index patient. One silent variation (p.V195) was predicted by FSPLICE to establish a novel splice donor site. In addition, all novel intronic variations were subjected to FSPLICE, revealing 1 variation (c.1161–70G>A) possibly activating a cryptic splice acceptor site. Both p.V195 and c.1161–70G>A were also absent in 800 control individuals. Pathogenicity of these mutations could not be supported by segregation analyses due to the absence of a positive history of PD in the families of 4 carriers. A niece of the p.M607V carrier suffers from PD, but DNA of this individual was not available for genetic testing. All carriers presented with classic PD (Supplementary Table 2) lacking atypical features and signs of cognitive impairment. In addition we identified 1 novel variation in the 3′ untranslated region, though not located in a known micro RNA binding site. Dosage analysis of *VPS35* did not identify whole gene copy number variations (CNVs) in the Flanders-Belgian LBD patient cohort.

4. Discussion

Genotyping of *VPS35* p.D620N in 4812 multiethnic PD patients suggested a prevalence of 0.14% (Vilarino-Guell et al., 2011; Zimprich et al., 2011). In accordance with this estimated prevalence we did not identify p.D620N in 677 Flanders-Belgian LBD patients. Remarkably, we did identify 5 rare novel variations at evolutionary strongly conserved positions, that likely altered either protein sequence or splice forms and were absent from 800 control individuals, suggesting that they are either associated with disease or are rare benign polymorphisms. The pathogenic role of p.D620N was supported by cosegregation with disease in large PD pedigrees and by in silico predictions (PolyPhen-2, SNAP, and SIFT) and molecular dynamics simulations (Vilarino-Guell et al., 2011; Zimprich et al., 2011) in addition to its absence in control individuals and strong amino acid conservation. In our case, absence of apparent familial history in 4 index carriers argued against a causal effect. Furthermore, in silico predictions for p.H599R and p.M607V did not support a damaging effect. Although Polyphen-2 and SIFT suggested a potential deleterious effect for p.I560T, the variation was also observed in 1 elderly unaffected sibling (AAI, 89 years old) of the carrier (AAO, 68 years old). Together, our current computational and genetic data are insufficient to confirm a causal relationship between the *VPS35* variations and PD development. Only 190 multiethnic familial patients were analyzed for *VPS35* CNVs, though with negative results (Vilarino-Guell et al., 2011). Our dosage analyses on approximately 600 LBD patients did also not identify a *VPS35* multiplication or deletion concluding that currently, there is no evidence for the existence of PD related *VPS35* CNVs. Deficiencies in the retromer sorting pathway have also been associated with late-onset Alzheimer's disease (AD) (Small et al., 2005). In our study, we did not find a *VPS35* variation underlying the Parkinson disease with dementia or dementia with Lewy bodies phenotype. Also, carriers of the pathogenic *VPS35* p.D620N did not present with cognitive deficits (Vilarino-Guell et al., 2011). In conclusion, the currently available data do not support a major role for *VPS35* genetic variability in LBD in the Flanders-Belgian study cohort.

Disclosure statement

The authors disclose no conflicts of interest.

Clinical and genetic studies described in this manuscript

were approved by the medical ethical committee of the Hospital Network Antwerp, the University Hospital of Antwerp, the University of Antwerp and the University Hospitals of Leuven, Belgium.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2012.01.006.